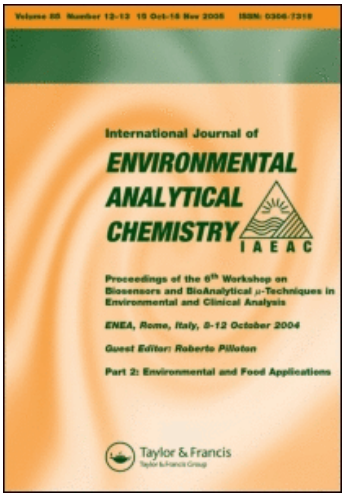

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Polynuclear Aromatic and Polycyclic Aliphatic Hydrocarbons in Sediments from the Atlantic Outer Continental Shelf†

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Surface sediments from the Atlantic Outer Continental Shelf between New Jersey and Virginia were analyzed for their hydrocarbon composition. Benzene fractions of sediment extracts were found to contain a variety of PNA's that in general were superimposed on complex mixtures of petrolic and/or biogenic origin. Concentrations of individual PNA's typically were at the ng/g level referred to dry weight. It is likely that these PNA's derive from pyrogenic input.

All hexane fractions contain pentacyclic triterpanes and triterpenes. The (17α)-hopane and its homologues are of special interest because of their high concentration (relative to other triterpanes) as well as their possible relation to the presence of petroleum. Absolute concentrations of (17α)-hopanes are of the order of ng/g (dry weight). The question of biological relevance of (17α)-hopanes as an indicator of petroleum is discussed briefly.

KEY WORDS: Sediments, hydrocarbons, polynuclear aromatics (PNA's), hopanes, triterpenes, GC–MS.

I. INTRODUCTION

During the past two years, the Virginia Institute of Marine Science has been engaged in an interdisciplinary study of the Outer Continental Shelf along the Mid-Atlantic Seaboard, which has resulted in a large body of physical, chemical and biological information.

This presentation concerns itself only with a very limited aspect of the

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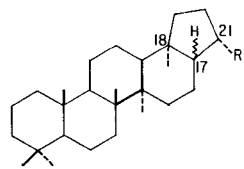
chemical work: the polynuclear aromatics (PNA's) and polycyclic aliphatics (PCA's) encountered in surface sediments. Only samples that have been analyzed by GC and GC-MS are discussed (about 10% of all samples extracted). The presence of a complex mixture of PNA's in some sediments was first realized by Giger and Blumer.[1] These authors concluded that many of the observed PNA's were likely to be of pyrolytic origin rather than petroleum. This hypothesis was further strengthened in studies by Youngblood and Blumer[2] and by Hase and Hites.[3] The latter authors also investigated a possible biogenic origin of such structures[4] as suggested earlier by a number of researchers (for references on this subject see Ref. 4), and concluded that such an origin was unlikely. The subject of PNA's in the aquatic environment was again discussed in a recent publication by Giger and Schaffner.[5]

PCA's of the triterpane family were long known by geochemists to occur in some geologic formations and in crude petroleum. However, only recently has the more academic interest in these complex hydrocarbon structures, in particular some of the pentacyclic triterpanes, also become of concern to the environmental chemist.[6] The (17α, 21β)-hopanes are of particular interest (Figure 1). Much of the fundamental work for the



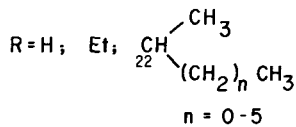


FIGURE 1 Structure of hopanes. In the (17α)-form, the *H* at position 17 is *cis* to the *R* group at position 21. For the (17β)-form it is *trans*. $R=H$ is trisnor-hopane, $R=$Et is norhopane, $R=i-$Pr is hopane and $R=i-$Bu is homo-hopane. Two stereomeric forms are possible only for $n>0$.

identification and the application of (17α,21β)-hopanes as characteristic indicators of petroleum has come from laboratories in Strasbourg[7,8] and Bristol[8,9]. Of special significance was the discovery that the (17α, 21β)-hopanes are the stable end products of a number of biogenic (17β, 21β) precursors[10]. The formation of (17α, 21β) epimers is proposed to take place during maturation.

II. METHODS

a. Sampling

Samples were taken in a Smith–MacIntyre grab. The grab was washed with methanol and toluene in between stations and was washed with ethanol between grabs. Approximately 1 kilogram of sediment was removed from the central, undisturbed portion of each grab to a depth of 5 cm, using an ethanol washed stainless steel scoop. Additional precautions taken to avoid contamination from the ship included covering the grab with a Teflon® shroud when not in use, and dispersing of the surface slick (when present) with a stream of water before deployment of the grab. Sediments were placed directly in wide mouth glass bottles with Teflon® lined caps, and quick frozen by solid CO_2 prior to storage at $-20°C$.

b. Extractions

The frozen sediments were thawed, washed with distilled and solvent extracted H_2O to remove salts, placed in pre-cleaned stainless steel trays and then freeze-dried for around 24 hours. The lyophilized sediments were spiked with internal standards and then refluxed 14 hours with a 3:7 toluene–methanol azeotropic mixture; the solvent was changed once after 7 hours. The combined total extracts were rotary evaporated to 50 ml and then saponified with 0.5 M KOH in 1:1 methanol–water for 4 hours under reflux. The non-saponifiable fraction was extracted with hexane. Sulfur was removed by activated copper powder. After drying overnight with anhydrous Na_2SO_4, the hexane extract was reduced to 3 ml by rotary evaporation, transferred to a 15 ml centrifuge tube, and then evaporated gently under an N_2 stream to 1 ml (for more details, see Figure 2).

c. Column chromatography

Columns were standard 10×300 mm with coarse glass frit, packed with a hexane slurry of silica gel (100–200 mesh Bio-Sil®-A) activated at 235°C for 16 hours. The gel was settled with a vibrator to a height of 175 mm so that the hexane eluting flow rate was less than 2 ml/min. A layer of sea sand (Fisher reagent S-25) 1–5 mm thick was applied to the top of the column.

The concentrated extract was applied to the prewashed column and eluted with hexane. After discarding the first 5 ml of hexane, 13 ml of the eluate were collected (hexane fraction); elution was continued with 40/60 (v/v) of benzene–hexane, of which 30 ml were collected (benzene fraction). The hexane fraction was evaporated under nitrogen to 0.2 ml.

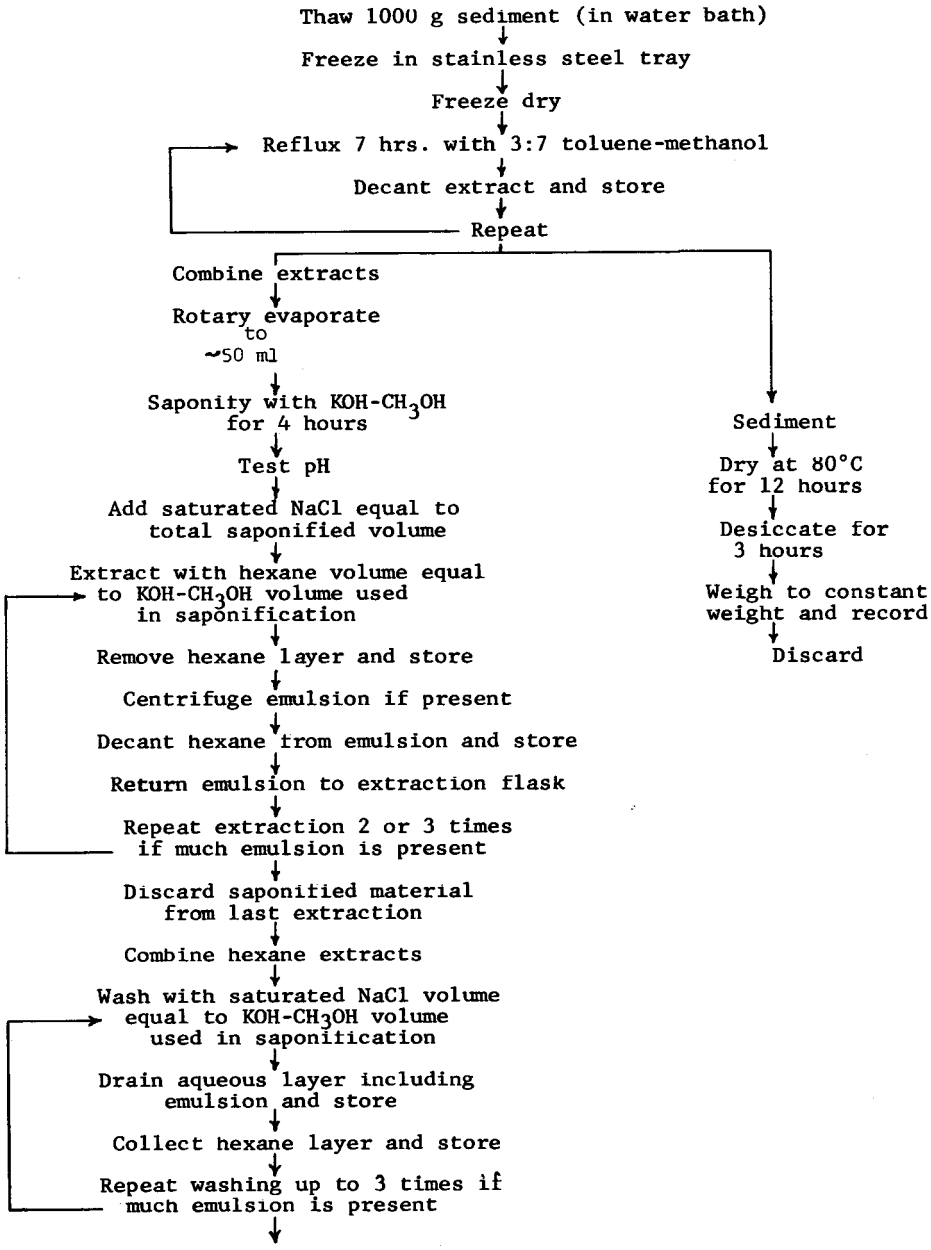


Fig 2 cont. Bieri

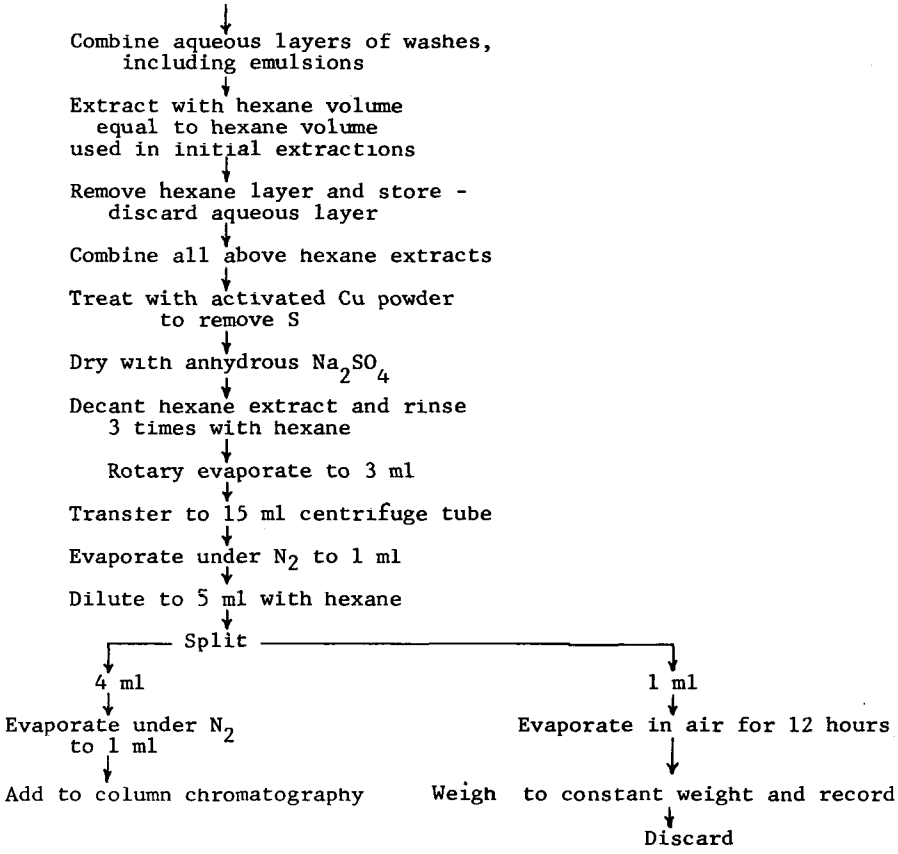


FIGURE 2 Laboratory procedures for isolation of sediment hydrocarbons.

The benzene fraction was evaporated to 1 ml, made up to 5 ml with hexane, and then evaporated to 0.2 ml.

d. Gas chromatography

A glass capillary system was used for gas chromatography (GC) of all samples reported here. Gas chromatographs were modified Varian 2700's with direct data outputs to strip chart recorders and a Hewlett–Packard 3352B laboratory data system.

Conditions of GC were:

Injection: Splitless[11]
Injector temperature: 270°C

Detector temperature: 265°C
Column inside diameter: 0.28 mm
Column length: 20 m
Liquid phase: SE-52
Column flow: ca. 5 ml/min He carrier gas
Column temp. program: 50 to 240°C at 6 C/min
(then hold until C_{32} detected).

Glass capillary columns were coated in this laboratory by the method of Grob and Grob[12].

The data system provided a tabulation of retention times and corresponding peak areas for each gas chromatogram. Each day a qualitative and quantitative aliphatic hydrocarbon standard, composed of the compounds listed in Table I, was run. The standard was used to establish GC response curves and to calibrate retention times of hydrocarbon peaks in aliphatic gas chromatograms, and to define the retention index scale for gas chromatograms of aromatic hydrocarbons for quantitative analyses.

Direct identification of aromatic peaks by Kovats index[15] has given inconsistent results[16]. Therefore, the aromatic hydrocarbons were instead assigned on the basis of GC-MS analyses.

e. Gas chromatography-mass spectrometry (GC-MS)

With SCOT, PLOT or packed columns, superimposition is the limiting factor in the analysis of complex mixtures, since this results in mass spectra that may be difficult to interpret or recognize. Wall coated glass capillaries eliminate some of these problems, but not all (even the best capillaries often contain peaks composed of several different compounds[13]). Their use is therefore important for the identification of compounds in complex mixtures. All analyses were carried out on a DuPont® 492-B mass spectrometer, interfaced to a Varian® 2740 gas chromatograph that was modified for glass capillaries in our laboratory. The column effluent was admitted via a Henneberg type interface[14] into a platinum capillary that led directly into the mass spectrometer source. Ionization occurred by electron impact at 70 eV. The system was operated in a continuous scan mode with 2.8 sec repetition rate and 1 sec/decade scanning speed. Resolution, $R/\Delta R$, was adjusted to approximately 1000. Data transfer and storage was accomplished by a DuPont® 91-A data system with software to allow retrieval of data in several convenient forms (reconstructed chromatograms, mass chromatograms, and mass spectra). Although the data system is also capable of compound identification, we found manual interpretation of spectra more reliable and quicker. Since mass spectral information in most cases must be supplemented by

TABLE I

Composition of aliphatic hydrocarbon standard

Compounds	Concentration mg/l
n-C_{10}	1.87
n-C_{11}	1.84
n-C_{13}	1.92
n-C_{14}	1.79
n-C_{15}	1.81
n-C_{16}	2.81
n-C_{17}	2.45
Pristane	2.81
n-C_{18}	2.66
n-C_{19}	2.64
n-C_{20}	2.74
n-C_{21}	4.97
n-C_{22}	2.95
n-C_{23}	6.31
n-C_{24}	3.00
n-C_{25}	2.50
n-C_{26}	4.38
n-C_{28}	4.32
n-C_{29}	3.38
n-C_{30}	4.78
n-C_{31}	4.51
n-C_{32}	7.74

Injections were approximately 2 μl of standard

retention data from gas chromatography for the identification of structural details, all identified compounds were plotted as a function of a normalized scan number. In a second step, the scan number was replaced by a relative retention index based on *n*-alkanes[15] for hexane fractions and on unsubstituted aromatics[16] for the benzene fractions. Compound identifications initially based on mass spectra were then confirmed by checking their coincidence with retention data from our laboratory or from published information. For the identification of members of the hopane family we relied on published facts about their geochemistry[7,17] as well as mass spectral information[9,18,19,20]. A branched-cyclic fraction of a Libyan oil was used as a secondary standard.

III. RESULTS AND DISCUSSION

a. Polynuclear aromatics

As judged from previous experience in the analysis of crude and refined oils[21,22] it was quite obvious that the benzene fractions from our sediment

extracts contained many PNA's that were of non-petrolic origin, but were similar in their composition to extracts from air particulates[23-27]. A summary of selected unsubstituted PNA's, normalized to the concentration of phenanthrene is found in Table II. We present the data in normalized form for two reasons. First, we consider them semi-quantitative, mainly because of the possible presence of superimposed non-aromatic compounds that are easily missed in the mass spectrum. Second, irregularities in their distribution are highlighted. For comparison, data of Giger and Schaffner[5]—in the following referred to as G&S—from aqueous environments have been included in the last three columns. Three typical sample chromatograms encountered in these sediment extracts are shown in Figures 3–5, and the sample location is found in Figure 6. Figure 3 shows the chromatogram of a sample that mainly contained

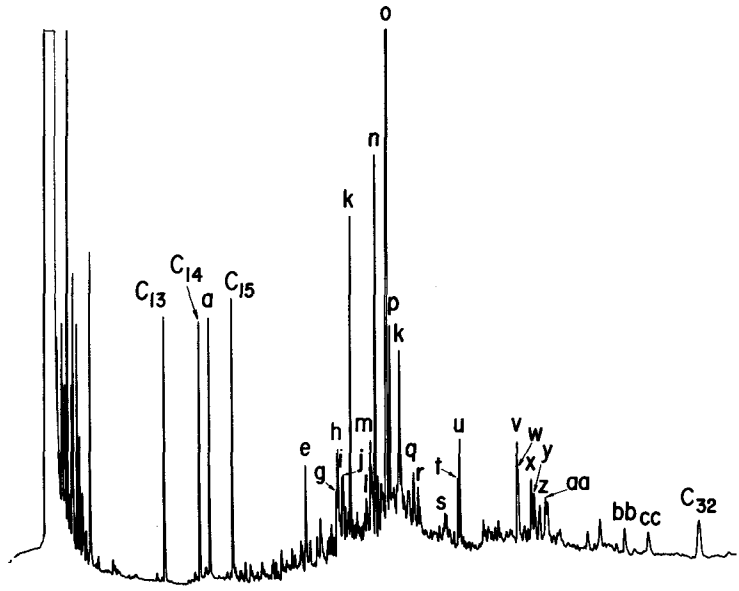


FIGURE 3 Representative chromatogram of an extract containing mainly PNA's of possible pyrogenic origin. Sediment, benzene fraction, cruise 03, station A4-2. Labeled peaks are: (a) hexamethylbenzene (spike), (e) phenanthrene, (g) 3-methylphenanthrene, (h) 2-methylphenanthrene, (i) 9-methylphenanthrene, (j) 1-methylphenanthrene, (k) not aromatic, (l) C_2-phenanthrene, (m) C_2-phenanthrene, (n) fluoranthene, (o) pyrene (spike), (p) C_2-4H-cyclopenta(d, e, f)phenanthrene, (q) benzo(a)fluorene, (r) benzo(b)fluorene and/or 2-methylpyrene, (s) benzo(g, h, i)fluoranthene, (t) benzo(a)anthracene, (u) chrysene/triphenylene, (v) benzo(j)fluoranthene, (w) benzo(k)fluoranthene, (x) benzo(e)pyrene, (y) benzo(a)pyrene, (z) perylene, (aa) cholestadiene, (bb) dibenzofluoranthene, (cc) benzo(g, h, i)perylene. Aliphatic spikes and hexamethylbenzene were added just prior to G.C. analysis.

TABLE II

Absolute concentration of phenanthrene (in ng/g). Concentrations of other PNA's (characteristically found in particulate air samples) are relative to phenanthrene.

	Outer Atlantic Shelf samples†											Giger & Schaffner		
	1A3-2	3A2-1	3A4-2	3B2-2	2G1-2	2K5-5	2L1-1	3 Int.-1	3 Int.-6	3 Int.-8	3 Int.-9	Lake	River	River Particles
Concentr. of phenanthrene	5.2	2.2	1.7	0.34	7.6	1.1	0.63	1.0	5.1	0.24	0.38	340	210	1600
Rel. conc. of:														
Anthracene	0.22	0.12	0.14	0.18	0.17	0.20	0.33	0.23	0.27	0.29	0.16	0.09	0.1	0.2
Fluoranthene	2.0	6.9	3.0	1.7	2.2	2.4	1.5	8.1	2.1	5.5	5.0	1.2	1.9	3.6
Benzo(a)fluorene	1.0	1.0	0.6	1.5	0.3	1.2	0.9	0.8	0.4	0.9	0.7			
Benzo(g, h, i)fluoranthene	0.4	2.3	0.5	1.9	—	1.5	—	0.7	—	—	0.2			
Benzo(a)anthracene	0.7	3.0	2.1	1.3	0.8	1.6	0.6	2.2	1.8	3.2	1.7			
Chrysene/triphenylene	1.1	3.4	2.1	1.4	1.0	2.9	0.6	3.2	1.5	3.5	2.7			
Benzo(j + k)fluoranthenes	2.1	12.5	5.4	3.4	1.9	9.8	0.8	11.8	1.8	11.3	7.6			
Benzo(e)pyrene	1.1	4.3	1.5	0.8	0.6	2.2	0.5	6.5	0.9	6.4	4.0	0.6	0.7	2.2
Benzo(a)pyrene	1.0	2.6	2.1	0.5	1.0	1.7	0.3	3.2‡	1.2	1.8	1.1	0.5	0.9	2.4
Perylene	0.4	12.7	—§	1.8	0.3	3.2	0.9	—§	1.2	9.5	1.0	0.1	0.1	0.4

†For sample location, see Figure 6.
‡Olefin superimposed.
§Interference with cholestadiene.

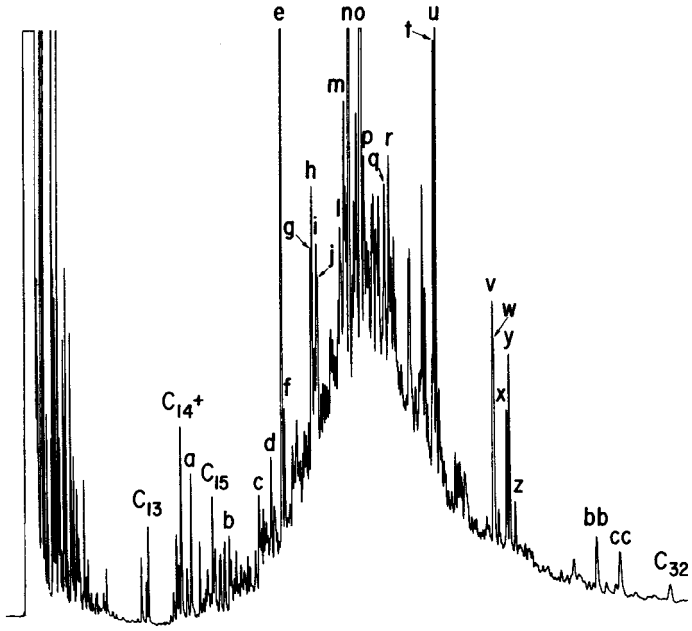


FIGURE 4 Representative chromatogram of an extract that is clearly contaminated by relatively fresh petroleum. Sediment, benzene fraction, cruise 02, station G1-2. Labeled peaks are: (a) hexamethylbenzene (spike), (b) fluorene, (c) C_4-naphthalene + C_5-naphthalene, (d) C_4-naphthalene, (e) phenanthrene, (f) anthracene, (g) 3-methylphenanthrene, (h) 2-methylphenanthrene, (i) 9-methylphenanthrene, (j) 1-methylphenanthrene, (l) C_2-phenanthrene, (m) C_2-phenanthrene, (n) fluoranthene, (o) pyrene (spike), (p) C_2-4H-cyclopenta(d, e, f)phenanthrene, (q) benzo(a)fluorene, (r) benzo(b)fluorene and/or 2-methylpyrene, (t) benzo(a)anthracene, (u) chrysene/triphenylene, (v) benzo(j)fluoranthene, (w) benzo(k)fluoranthene, (x) benzo(e)pyrene, (y) benzo(a)pyrene, (z) perylene, (bb) dibenzofluoranthene, (cc) benzo(g, h, i)perylene. Aliphatic spikes and hexamethylbenzene were added just prior to G.C. analysis.

PNA's. The chromatogram in Figure 4 is clearly contaminated by oil and that of Figure 5 contains an abundance of unidentified compounds possessing non-aromatic structure (probably biogenic polyenes). These chromatograms give visual evidence that while the benzene fractions of Outer Continental Shelf sediments always contain some background, the unsubstituted PNA fraction is easily recognized once the individual compounds have been identified. In comparing these chromatograms with those of G&S, it must be noted that G&S use a column chromatographic technique that specifically isolates PNA's.

Although the concentration of phenanthrene in our samples is approximately two orders of magnitude lower than G&S, the relative concentrations of fluoranthene, benzo(e)pyrene and benzo(a)pyrene in some

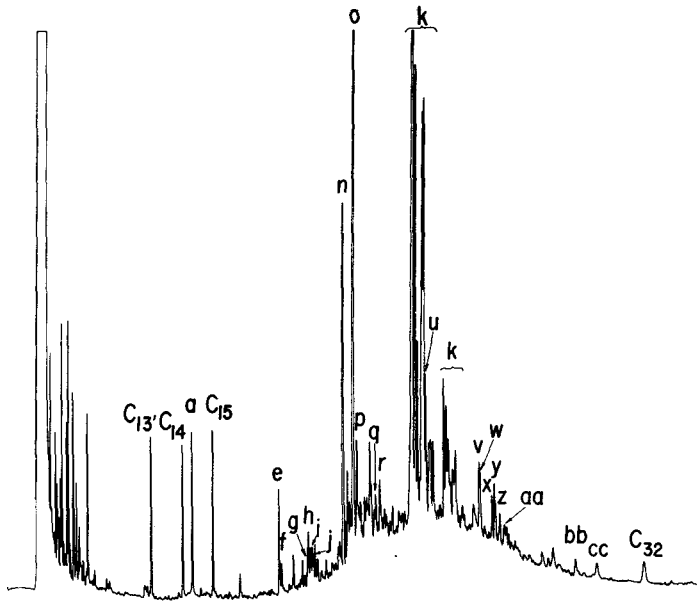


FIGURE 5 Representative chromatogram of an extract containing PNA's superimposed on a mainly olefinic background. Sediment benzene fraction, cruise 03. Intercalibration 6 (station A1). Labeled peaks are: (a) hexamethylbenzene (spike), (e) phenanthrene, (f) anthracene, (g) 3-methylphenanthrene, (h) 2-methylphenanthrene, (i) 9-methylphenanthrene, (j) 1-methylphenanthrene, (k) not aromatic, (n) fluoranthene, (o) pyrene (spike), (p) C_2-4H-cyclopenta(d,e,f)phenanthrene, (q) benzo(a)fluorene, (r) benzo(b)fluorene and/or 2-methylpyrene, (u) chrysene/triphenylene, (v) benzo(j)fluoranthene, (w) benzo(k)fluoranthene, (x) benzo(e)pyrene, (y) benzo(a)pyrene, (z) perylene, (aa) cholestadiene, (bb) dibenzofluoranthene, (cc) benzo(g,h,i)perylene. Aliphatic spikes and hexamethylbenzene were added just prior to G.C. analysis.

samples are very similar. Relative concentrations of perylene in our samples appear to be systematically higher, but an isomer of cholestadiene was found to elute close to the perylene. This interference was recognized in two samples (3A4-2 and 3Int-1), but may have been overlooked in the samples where perylene shows an unusually high concentration. Since cholestadiene is a biogenic compound, it seems reasonable to expect that its contribution is diminishing as the concentration of PNA's increases. The much lower relative perylene concentrations of G&S are thus not surprising. However, there is some evidence that perylene may not have a purely pyrolytic origin but may in part also be derived from biogenic precursors[28].

Samples such as 3A2-1, 3Int-1 and 3Int-8 appear to contain PNA fractions that are high mass in 252 isomers (e.g. perylene...) as well as

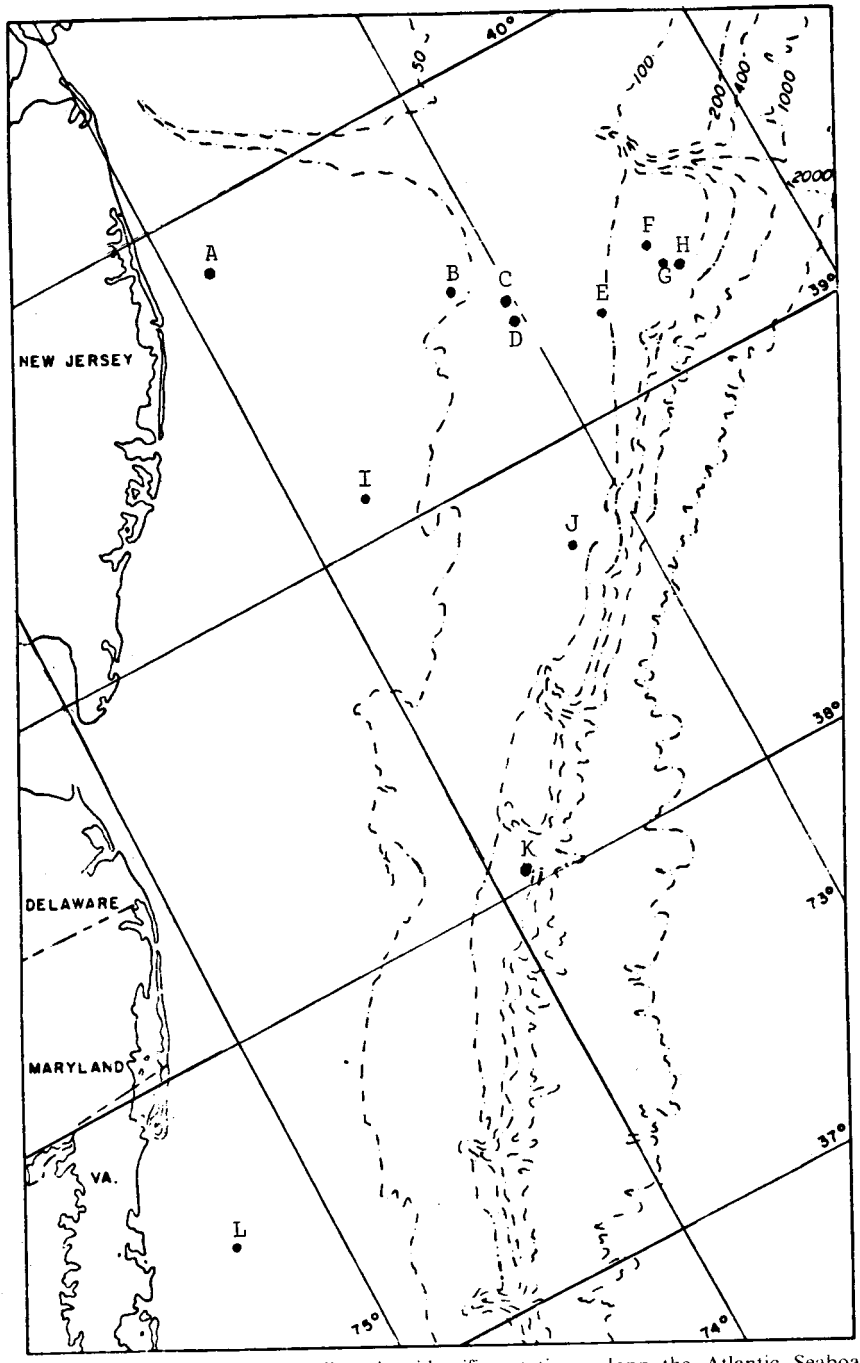


FIGURE 6 Sample location. The plot identifies stations along the Atlantic Seaboard between New Jersey and Virginia where sediments were collected. Samples are identified in the text by their cruise number and station name followed by a replicate number. The labelled stations are: (A) G1, (B) B4, (C) B2, (D) Int.-9, (E) Int.-6, (F) A2, (G) A3, (H) A4 and Int.-8, (I) D3 and Int.-1, (J) Int.-4, (K) K5, and (L) L1.

fluoranthene relative to G&S. This could be the result of compositional differences in the source providing the pyrogenic input, or it could derive from fractionation. Contamination by crude and refined oils—at least those that are familiar to us—would raise mainly the concentration of phenanthrene and thus decrease the relative concentration of all other PNA's listed in Table II. Of the seven samples that show fair agreement with G&S, three indeed have relatively high phenanthrene concentrations. A check of other indicators for oil (the presence of naphthalenes, and biphenyls; the presence of an unresolved complex mixture (UCM); in the hexane fraction, the carbon preference index (CPI)), however, gives contradictory results.

The CPI in sample 1A3-2 is consistent with the presence of petroleum, and so are the aromatic indicators (substituted naphthalenes and biphenyls, concentration of phenanthrene). Since the aromatic fraction of this sample also has a substantial UCM, there is little doubt that it is contaminated. All aromatic indicators including the UCM in sample 2Gl-2 strongly indicate petroleum contamination, but the CPI is the highest found in any of the samples in Table II. This can only be explained by the overpowering influence of a continental source of plant detritus. Sample 3Int-6, finally, had no identifiable evidence for 2-ring aromatics. A small UCM is present in the chromatogram, but mass spectrometric inspection suggests a predominantly olefinic composition. Additional evidence for the presence of biogenic compounds is found from resolved peaks. While the CPI of this sample is consistent with petroleum contamination, closer inspection shows low *n*-alkane concentrations and the possibility of superimposition. Under these circumstances, we are tempted not to attach too much weight to the CPI.

The evidence for the presence of a pyrogenic fraction in these surface sediments, despite its low concentration, is clear. Due to its low level and the column chromatography employed, it always is superimposed on hydrocarbons of different origin and is therefore more difficult to recognize than in samples analyzed by G&S, where PNA concentrations are about 2 orders of magnitude higher. Contamination with a relatively undegraded fresh oil is indicated in samples 1A3-2 and 2Gl-2, but with the exception of phenanthrene it does not appear to substantially affect the distribution of PNA's.

b. Polycyclic aliphatics

Hopanes of the (17α, 21β)-type were encountered quite regularly in the hexane fractions of Outer Continental Shelf sediments. Several as yet unidentified PCA's are also present and often are superimposed on the hopanes (Figure 7).

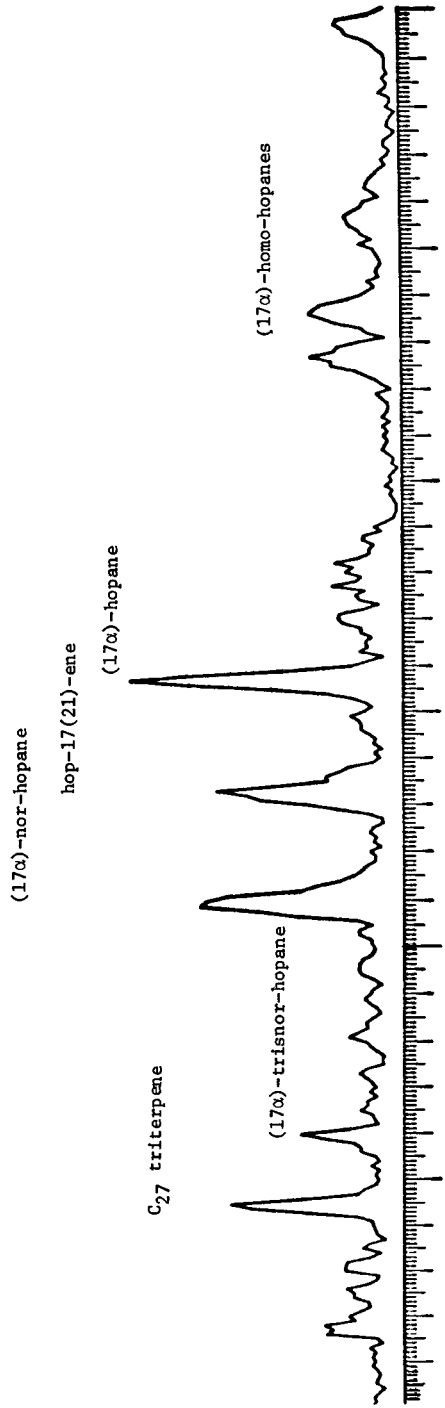


FIGURE 7 Reconstructed chromatogram of the m/e 191 fragment characteristic of hopanes and other pentacyclic triterpanes. Only the C_{27}-triterpene and the (17α)-hopane appear to be pure: all others indicate the presence of some superimposed compounds.

Trisnor-hopanes were confirmed in some samples, but available evidence was not conclusive in others. This homologue in general was present only in trace concentrations and in many samples was superimposed to another pentacyclic triterpane, which according to its mass spectrum, is probably the (17β)-epimer of trisnor-hopane. A C_{30}-triterpene with intensity similar to hopane was also commonly encountered. The mass spectrum of this unsaturated compound indicates that it is hop-17(21)-ene[20]. Finally, mass chromatograms of m/e 191 indicate the presence of another triterpene with 27 carbons (m.w. 368) whose structure could not be detailed further. Because the G.C.-M.S. runs were terminated after elution of the C_{32} *n*-alkane standard, no homologues past the R and S stereomers of homophane were identified.

Semiquantitative data on (17α)-hopanes, together with the CPI, are listed in Table III. The hopane concentrations on the average are quite

TABLE III

Concentration of (17α, 21β)-hopane and relative concentrations of homologue (17α)-hopanes[a]

Sample	C_{hopane}[b] ng/g	$C_{rel.}$ trisnor-[c]	nor-[d]	homo-#1	homo-#2	CPI[f]
1A3-1	1.5	0.5	0.9	0.3	0.5	2.7
1A3-2	2.3	0.4	1.0	—[e]	—[e]	1.4
1D3-5	3.3	0.2	0.9	—[e]	—[e]	2.0
2K5-5	2.4	0.3	1.0	0.6	0.6	2.3
3A4-2	2.5	0.4	0.8	0.4	0.5	2.0
3B4-2	2.3	trace	0.9	0.5	—[e]	1.1
3Int.-1	trace	0.2	1.0	0.4	—[e]	2.8
3Int.-4	1.4	0.4	0.9	0.4	0.5	1.2

[a] Calculated from ionic data.
[b] Concentration calculated from *n*-alkane standards.
[c] Mass-spectrometry suggests presence of both (17α) *and* (17β)-forms.
[d] Mass-spectrometry suggests several isomers superimposed.
[e] GC-MS run terminated before elution of this peak.
[f] Calculated for *n*-alkanes *n*-C_{22} to *n*-C_{30}.

low, of the order of ng/g, and approximately equal to the nor-hopane concentrations. The concentrations of the R and S stereomer of homohopane also are about equal, but only half of those for hopane and norhopane. The presence of two homohopanes with similar concentrations is characteristic of the (17α, 21β)-structure and indicates formation by maturation or thermodynamic equilibration[7,10]. The (17β, 21β)-form usually is encountered either as the R or S stereomer[10].

Trisnor-hopane also is less abundant than hopane or nor-hopane. Since mass spectral evidence suggests the presence of both (17α) and (17β) structures in a merged peak, the reported relative concentrations of trisnor-hopane should be considered as upper limits. In comparing the relative homologue concentrations, allowance must also be made for the fact that the relative contribution of the m/e 191 fragment to the total ion intensity has been assumed to be constant for different homologues. Although we realize that this is a crude approximation, a statistical evaluation of all mass spectra did not justify more refined treatment. The method of using ionic data was judged to be superior to a quantitation of individual homologue concentrations from gas chromatograms, which show evidence of non-triterpenoid peak superimposition in the region of nor-hopane and the homo-hopanes.

Aside from such details, the important fact remains that (17α,21β)-hopane structures are encountered in every sample, including those that show no other evidence for the presence of petroleum or, more generally, fossil fuels.

The origin of these (17α,21β)-hopanes is difficult to pinpoint without also investigating the hopanoic acids[6] and considering other sources. For example, most of the eroded material on the shelf is from Pliocene–Pleistocene formations which are in principle old enough to contain some (17α)-hopanes. Nevertheless, while we cannot exclude the possible contribution of such sources, we believe that a petrolic origin of the (17α)-hopanes incorporated in the Mid-Atlantic Shelf sediments is likely.

Of the two unsaturated triterpenoids, hop-17(21)-ene[20] (RI = 3013) elutes almost simultaneously with another compound that has a very similar mass spectrum. As suggested by Ensminger[7,20], hop-17(21)-ene is likely to derive from a biogenic precursor, diploptene. Such a biogenic origin could explain the larger range of variation of hop-17(21)-ene as compared to the (17α)-hopanes. The other triterpene (RI = 2865) has an even wider range of variation, but its variation is not correlated to hop-17(21)-ene. This requires further investigation.

Many other PCA structures are present in addition to those mentioned so far, but at much lower levels. Although some identifications were marginally possible by mass spectrometry (e.g., some (17β,21β)-hopanes), more complete information, especially on retention, is required to get a definitive idea about their structure.

The use of (17α,21β)-hopanes as indicators for the presence of petroleum without doubt is very powerful and sensitive. As far as the biologist and those interested in ecological aspects are concerned, however, there are some doubts about their relevance. The very refractory residuals of crude oils (formed after extensive weathering) are not likely to

have any direct effect on the biota[22]. The (17α)-hopanes are the most characteristic molecular fossils known today and because of their chemical inertness may persist after biologically more relevant indicators of petroleum have vanished. For example, note the lack of correlation between the hopane concentrations and the CPI in Table III. Unless toxic effects can be related to the presence (17α)-hopanes, they are of little consequence to those concerned about the health of the environment, especially at low levels. Nevertheless, the value of hopanes as organic geochemical tracers is unquestionable.

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